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AMMONIA STRESS INDUCED BIOCHEMICAL CHANGES IN LIVER AND BRAIN OF ALBINO RAT

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ABSTRACT

Ammonia is not just a waste product of nitrogen metabolism but involved for the synthesis of many compounds in the body like aminoacids, purines, pyrimidines, aminosugars, asparagines etc. Excess ammonia is excreted mainly as urea which is synthesized in the liver through urea cycle. Ammonia is a normal constituent of all body fluids but can become a toxicant under ammonia stress which leads to ammonia toxicity or Hyperammonemia. In this condition metabolic mechanisms are disturbed. The present study aims to examine the changes in biochemical parameters under ammonia stress. The LD50 of ammoniumsulphate after toxicity evaluation was 91.5mg/kg body weight. The sub lethal dose i.e1/5 of LD_{50}, which is 18.3mg of ammoniumsulphate /kg body weight, was injected intraperitonially to rats for one week treatment. The selected biochemical parameters like Total proteins, Soluble proteins, Structural proteins, Free amino acids and Proteases levels were estimated in Liver and Brain tissues of Albino rats by using standard methods. The changes in these biochemical components under ammonia stress will be discussed.

KEY WORDS: Ammonia stress, Total Proteins, Soluble and Structural Proteins, Free aminoacids, Proteases.

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INTRODUCTION

Ammonia is an important source of nitrogen and is required for amino acid synthesis. Nitrogenous waste results from the breakdown and catabolism of dietary and body proteins, respectively. In healthy individuals, amino acids that are not needed for protein synthesis are metabolized in various chemical pathways, with the rest of the nitrogen waste being converted to urea. Ammonia is important for normal animal acid-base balance. In the brain, the activity of glutamate dehydrogenase mediates ammonia production. After formation of ammonium from glutamine, α-ketoglutarate, a byproduct, may be degraded to produce two molecules of bicarbonate, which are then available to buffer acids produced by dietary sources. Ammonium is excreted in the urine, resulting in net acid loss. The ammonia level generally remains low (<40 mmol/L) due to the fact that most ammonia produced in tissue is converted to glutamine. Glutamine is also excreted by the kidneys and utilized for energy production by gut cells, which convert the nitrogen byproduct into alanine, citrulline, and ammonia, which are transported to the liver via the bloodstream. Ammonia enters the urea cycle in hepatocytes or is ultimately converted to glutamine (Walker. 2009 and Singh 2007) [1,2].Ammonia is toxic when present in high concentrations. Endogenous ammonia intoxication can occur when there is impaired capacity of the body to excrete nitrogenous waste, as seen with congenital enzymatic deficiencies. Patients with urea cycle defects (UCD), organic acidemias, fatty acid oxidation defects, bypass of the major site of detoxification (liver) (such as that seen in cirrhosis), Reye syndrome, post chemotherapy, or exposure to various toxins and drugs can all present with elevations in ammonia. Delayed diagnosis or treatment of hyperammonemia, irrespective of the etiology, leads to neurologic damage and potentially a fatal outcome, and thus it becomes a medical emergency when present (Ari Auron et.al.,2012)[3].Interests in studying ammonia toxicity in mammals arise from the fact that liver failure in human leads to the development of neurological abnormalities collectively referred to as hepatic encephalopathy, and ammonia is a key neurotoxin implicated in this condition (Häussinger and Schliess, 2008; Lemberg and Fernandez, 2009)[4-5] .More recent studies in mammals have implicated oxidative stress and mitochondrial permeability transition in the mechanism of ammonia neurotoxicity (Norenberg et. al., 2005; Jayakumar et. al., 2006; Reddy et al., 2009; Häussinger and Görg, 2010)[6-9].In animals, amino acids are obtained through the consumption of foods containing protein. Ingested proteins are then broken down into amino acids through digestion, which typically involves denaturation of the protein through exposure to acid and hydrolysis by enzymes called proteases. Some ingested amino acids are used for protein biosynthesis, while others are converted to glucose through gluconeogenesis, or fed into the citric acids cycle.The present study is aimed to understand the changes in protein metabolism in albino rat under ammonia stress. As liver and brain form two important metabolic centres for ammonia products and impact of ammonia, these two tissues were selected for the study.

MATERIALS AND METHODS

Healthy male wistar strain albino rat (150 ± 20 g) obtained from Indian institute of science, Bangalore were maintained in polypropylene cage under laboratory condition (temperature 34 ± 2^0 C light: dark=12:12h humidity 75%) and fed with standard laboratory chow (Hindustan lever limited, Bombay) and water was provided ad libitum.Toxicity of ammonium sulphate was evaluated according to Finney's method[10] (1975) and was found to be 91.5 mg/Kg body weight. The sub lethal dose i.e, 1/5 of LD_{50}, which 18.3 mg ammonium sulphate/Kg body weight was injected intraperitonially to rats for one week treatment. The sub lethal dose was selected to keep the animal under ammonia

stress but does not cause mortality.Healthy adult animals were divided into two groups containing six animals each. The first group of animals was considered as control, the second group of animals received the ammonium sulphate were considered as experimental. The control and experimental animals were sacrificed by cervical dislocation at the end of the treatment i.e, 7 days and liver and brain tissues were collected and stored in deep freezer at -20^{0}C and used for biochemical analysis.Total proteins, soluble proteins, structural proteins was assayed by method of Lowry et.al[11](1951), Free amino acids was assayed by the method of Moore and Stein[12] (1954), Proteases was assayed by the method of Davis and Smith[13] (1955). The results were subjected to statistical analysis. The experimental protocol was approved by the institutional animal ethics committee (IAEC). (Resolution Number: 06/2012-2013/(i)/(a)/CPCSEA/IAEC/SVU/PN-ASR/dt. 1.2.2012).

RESULTS

The changes in the levels of Total proteins, soluble proteins, structural proteins, free amino acids, enzyme levels of Proteases in brain and liver tissues of 7days ammonium sulphate treated albino rats are shown in Tables 1&2.Ammonium sulphate administration for 7 days has shown a decrement in levels of Total proteins(-17.16%),, Soluble proteins(-29.31%), Structural proteins(-27.20%) and increment in levels of Free amino acids(13.16%), and Proteases levels(4.68%) in brain tissue when compared to control as shown inTable:1.
Ammonium sulphate administration for 7 days has shown a decrement in levels of Total proteins(-22.36%), Soluble proteins(-33.86%), Structural proteins(-26.07%)and increment in levels of Free amino acids(+16.40%), and Proteases levels(+9.41%) in liver tissue when compared to control as shown inTable:2.

Table 1

Changes in the Total proteins, soluble proteins, structural proteins, Free amino acids, Proteases levels in brain tissue of control and ammonium sulphate treated albino rats

Parameter		Control	Experimental
Total protein (mg/gm wet wt of tissue)			
	Mean	75.385	62.4483
	S .D	± 0.3368	± 0.4472
	% change		(-17.16)
	t-test		P< 0.001
Soluble protein (mg/gm wet wt of tissue)			
	Mean	44.5464	31.4856
	S.D	± 0.5762	± 0.3098
	% change		(-29.31)
	t-test		P<0.01
Structural protein (mg/gm wet wt of tissue)			
	Mean	31.3848	22.8456
	S.D	± 0.3352	± 0.308
	% change		(-27.20)
	t-test		P<0.01
Free amino acids (μ moles of tyrosine/gm wet wt of tissue)			
	Mean	68.7067	78.1053
	S.D	± 0.9653	± 1.54305
	% change		(+13.16)
	t-test		P<0.05
Proteases (μ moles of tyrosine/mg protein/hr)			
	Mean	1.2438	1.8264
	S.D	± 0.0724	± 0.1759
	% change		(+4.68)
	t-test		P<0.05

Table 2

Changes in the Total proteins, soluble proteins, structural proteins, free amino acids, Proteases levels in liver tissue of control and ammonium sulphate treated albino rats

Parameter		Control	Experimental
Total protein (mg/gm wet wt of tissue)			
	Mean	96.6024	74.998
	S.D	± 0.4070	± 0.34897
	% change		(-22.36)
	t-test		P< 0.001
Soluble protein (mg/gm wet wt of tissue)			
	Mean	57.5496	38.0592
	S.D	± 0.3016	± 0.2568
	% change		(-33.86)
	t-test		P< 0.01
Structural proteins (mg/gm wet wt of tissue)			
	Mean	37.5047	27.726
	S.D	± 0.6164	± 0.2783
	% change		(-26.07)
	t-test		P< 0.01
Free amino acids (μ moles of tyrosine/gm wet wt of tissue)			
	Mean	75.2051	87.5412
	S.D	± 1.1557	± 1.1431
	% change		(+16.40)
	t-test		P< 0.001
Proteases (μ moles of tyrosine/mg protein/hr)			
	Mean	1.3732	1.8264
	S.D	± 0.0087	± 0.3295
	% change		(+9.41)
	t-test		P< 0.05

DISCUSSION

Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells. Proteins are the molecular instruments through which genetic information is expressed (Nelson and Cox, 2005) [14].Proteins can be informally divided in to three main classes, which correlated with typical tertiary structures as globular proteins, Fibrous proteins and membrane proteins. Almost all globular proteins are soluble and many are enzymes. Fibrous proteins are often structured such as collagen, the major component of connective tissue or keratin, the protein component of hair and nails. Membrane proteins often serve as receptors or provide channels for polar or charged molecules to pass through the cell membrane (Kensal Van hold and Mathews 1999)[15].Proteins are the primary structural and functional polymers in living systems. Proteins are being the most important organic constituents of organs: Their role in the compensatory mechanism of animals can be accepted during stress conditions. The sub lethal dose of ammonium sulphate gave a decrement of Total proteins, soluble proteins and Structural proteins probably due to ammonia stress. Generally the breakdown of proteins dominates over synthesis under enhanced proteolytic activity (Murray et al., 2007)[16]. Proteins being the most important organic constituents of organs, their role in the compensatory mechanism of animal can be accepted during stress conditions (Singaraju et al., 1991)[17].Similar results are reported that ammonia stress conditions in fish resulted in decreased Total proteins, soluble proteins and Structural proteincontents (Hari 2010)[18].The Total proteins also gave decrement in rat

tissues in ammonia stress conditions (Sujatha 2011)[19]. The Total proteins decrement in ammonia stress condition was also reported in brain tissue of cockroach (Kishore et.al 2010)[20]. The oxidation pathway starts with the removal of amino group by a transaminase the amino group is then feed into the urea cycle (Brosnana 2000) [21]. The product of transamidations is a keto acid that enters the citric acid cycle. Glucogenic amino acid can also be converted into glucose, through gluconeogenesis (Young and Ajami, 2001)[22].The amino acids released during protein degradation due to activation of proteolysis will once again return to the amino acid pool and thus the free amino acids are the currency through which protein metabolism operates showing interdependence of both amino acids and proteins (Murray et al., 2007)[16]. The sub lethal dose of ammonium sulphate gaves an increment of free amino acids levels probably due to ammonia stress. Several authors reported increased free amino acid contents in different animals. Increased free amino acids in ammonia stress condition in fish (Hari 2010)[18] and cockroach(Kishore et.al 2010)[20] have been reported. A similar increase in free amino acid has been reported in albino mice in Aluminium acetate stress (John Sushma 1999)[23] and Cypermethrin stress condition in *Cirrhinus mrigala* (Neelima et.al 2011) [24].The increment in protease activity along with increase free amino acids supports the decrement in protein levels. Ammonia stress seems to result in protein breakdown.Hydrolysis of proteins is quite a common phenomenon where in proteases split proteins step wise into amino acids. The proteases are acidic natural and alkaline in nature. The aminoacids formed by protein degradation, on one hand, serve for the synthesis of required proteins on the other hand useful for meeting the necessary energy demand.The sub lethal dose of ammonium sulphate gave increment of protease levels probably due to the ammonia stress condition. The changes in protease activity indicate the change in energy cycle. Several authors reported increased protease contents in different animals. The proteases are increased in toxic condition. The increased protease activity in albino mice was reported (John Sushma 1999)[23]. A similar increase in Protease levels in Cypermethrin stress condition in *Cirrhinus mrigala* (Neelima et.al 2011)[24] was reported. Ammonia stress in albino rat seems to result in protein utilization and affect protein catabolism rather than anabolism. Further study is focused in understanding the mechanism of the pathways involved in protein metabolism.

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